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ALLELOPATHIC EFFECT OF *RHAZYA STRICTA* PLANT RESIDUE ON *RAPHANUS SATIVUS* (RADISH)

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Abstract

This study was designed to investigate the allelopathic potential of *Rhazya stricta* on *Raphanus sativus* using laboratory bioassay and greenhouse pot experiment. In laboratory bioassay, aqueous extract of *R. stricta* showed inhibitory effect on *R. sativus* seedling growth particularly at the high concentrations. The germination percentages were not significantly affected. Results in greenhouse pot experiment showed that the residue of *R. stricta* showed inhibitory effect on root length, dry weight and root to shoot length ratio of *R. sativus* especially at the high concentrations at different ages. The *Rhazya* residue showed positive effects on the photosynthetic pigments of *R. sativus* particularly on the carotenoids and chlorophyll a/b ratio at different ages. A significant increase in nitrogen content of *R. sativus* including total amount of free amino acid, soluble and insoluble nitrogen and crude protein was prominent at the early growth stage especially at the high concentrations. The *Rhazya* residue inhibited the contents of soluble nitrogen of *R. sativus* in the late growth stage. The results of RAPD-DNA profiles showed significant effect of *Rhazya* residue on *R. sativus* plant from where variation in band intensity, disappearance of bands, and appearance of new PCR product.

Keywords: allelochemicals**,** aqueous extract, photosynthetic pigments, RAPD-DNA profiles, residue.

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Introduction

Plants live association groups depending upon the ecological requirements; they have generally similar structural and morphological adaptations. Whenever two or more plants occupy the same niche in nature, they compete with each other for various life support requirements (Khan *et al*.,2011a, p. 81). Allelopathy refers to the beneficial or harmful effects of one plant on another one, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems. In agroecosystems, allelopathic effects between living weeds and crops, crops in mixtures, plant straw residue and succeeding crops during decomposition of residue are also well documented. Allelopathy is expected to be an important mechanism in the plant invasion process because of the lack of co-evolved tolerance of resistant vegetation to new chemicals produced by the invader. This phenomenon could allow the new introduced species to overlook natural plant communities (Khan *et al*., 2011b, p. 6392).

Rhazya stricta Decne, (Apocynaceae) is a perennial plant locally known as Harmal. It is widely distributed throughout Western Asia from Yemen to Arabia, to the North West Province of India and abundantly found in various regions of Pakistan (Baeshin *et al*., 2009, p. 986). *R. stricta* like other plants is competing with the main crops for nutrients and other resources and hamper the healthy growth of crops ultimately, reducing the yield both qualitatively and quantitatively (Mutawakil., 2012, p.11). (Al-Yahya *et al*.,1990 , p. 123) have reported the presences of alkaloids, glycosides, triterpenes, tannins and volatile bases in the leaves of this plant.

Raphanus sativus (radish) is a globally edible root and leaf vegetable. Radish is rich in ascorbic acid, folic acid, and potassium. It is also a good source of vitamin B6, riboflavin, magnesium, copper and calcium, *Raphanus sativus* contains flavonoids, saponins, tannins, glycosides, steroids and alkaloids.(Jan & Badar., 2012, p. 23).

No previous studies reported on the tolerance of *Raphanus sativus* to the allelopathic effect of *Rhazya* plant. However, there are many studies on the effect of *Rhazya stricta* as tested on seed germination of some other species (Khan *et al*., 2011b, p. 6391). The present study was conducted to explore allelopathic potential of *R. stricta* on ecophysiology of *R. sativus* in laboratory bioassay and greenhouse pot experiment.

Material and Methods

Plant materials

Plant material of *Rhazya stricta* was collected from its natural habitats in central Saudi Arabia. The plants were air dried, then ground into a fine powder and stored in refrigerator until used. The seeds of radish were obtained from the Agricultural Research Center, Vegetables Department, Egypt.

Preparation of Rhazya extract

Aqueous extracts of *Rhazya stricta* were prepared by shaking dry powdered tissue with distilled water for 24 hours at room temperature. Mixture was filtered through a suction filtration. The clear supernatant was brought to the original volume with distilled water to obtain the extract concentrations 0.05, 0.1, 0.5, 1, 2, 3, 4, 5 % (w/v). These water extracts were used in the bioassay tests.

Bioassay tests

Effects of *Rhazya* extracts on seed germination and seedling growth of *Raphanus sativus* were performed in the laboratory in covered glass Petri dish (9cm diameter) lined with one layer filter paper. In every dish10 radish seeds and 10 ml of the test extract were used. Distilled water was applied in the control treatment. The dishes were incubated in a dark growth chamber, at room temperature. Four replicates per treatment were used. Tests were terminated after 10 days. The final germination was calculated as percentage of control. The radical and plumule lengths of the seedlings were measured. The samples were dried to constant weight in an oven at 80 ^{o}C to obtain the dry weight. Root/shoot length and weight ratios were calculated.

Pot experiment

A greenhouse pot experiment was conducted to assess the possible inhibitory or stimulatory effects of *Rhazya* plant powder on *Raphanus sativus* plant. Pot experiment was carried out in plastic pots (13 cm in diameter and 14 cm in depth), each containing 2 kg of clay soil. The pots were divided into 8 groups, each was 12 pots, one was left without treatment as control and the other seven groups were treated with *Rhazya* residues. The fine ground shoot powder was incorporated into the upper soil layer with 2 cm depth that finally gave the percentages of 2, 4, 6, 8, 10, 12 and 16% (w/w). Ten healthy *R. sativus* seeds of uniform size were sown at 1 cm soil depth and the seedlings were thinned to 5 plants per pot after emergence. Plants were irrigated with tap water, and soil was kept at field capacity, along the whole experimental period, using weighing procedure. Pots were placed in an open greenhouse under natural conditions during March to April months. The plants, at the vegetative stage, were harvested after 30 days from sowing, then washed thoroughly with tap water and divided into root and shoot systems for measurement of growth criteria. Lengths of the main root and shoot, and their root/shoot length ratio were calculated. The samples were oven dried to a constant weight at 80^{o}C for dry weight measurements.

Photosynthetic Pigments

Pigments were extracted from fresh *Raphanus sativus* shoot with 100% acetone following the method used by (Fadeel,. 1962,p 130), then measured and calculated according to (Sestak *et al*., 1971, p. 682).

Extraction and Determination of Nitrogen

Total nitrogen was determined in plant powder after the acid digestion with 1 ml 50% H_2SO_4 and 1 ml 30% perchloric acid, using Bertholet reaction (Chaney and Marbach,. 1962, p 130). Soluble nitrogen were extracted from the dried *Raphanus sativus* shoot tissue with 10% trichloroacetic acid (TCA) and the remaining dried residue was acid digested to obtain the insoluble components. Total amount of free amino acids was estimated in the TCA extract as amino-N (Russell, 1944). Multiplying the total organic nitrogen by 6.25 estimated the crude protein (AOAC.,

1995).

Detection of DNA polymorphism using RAPD technique DNA extraction

DNA was isolated from 50 mg of plant material using Qiagen Kit for DNA extraction by a modified CTAB method (Doyle & Doyle., 1990, p. 13). The extracted DNA was dissolved in 100 µl of elution buffer. The concentration and purity of the obtained DNA was determined by using "Gen qunta" system-pharnacia Bio-teck. The purity of the DNA for all samples was between 90-97%. Concentration was adjusted at 6 mg/µl for all samples using TE buffer PH 8.

RAPD analysis

A total of 10 (10 mer) oligonucleotide random primers were used for RAPD analysis (Table1). DNA amplification reactions were performed in 25 µl reaction mixture consisting of 1 unit of Tag DNA polymerase, 0.2 mM dNTP, 1x PCR buffer, 3 mM $MgCl_2$, 10 Pmol of each primer and approximately 50 ng of the extracted genomic DNA. Amplification reactions were carried out using PCR unit II Biometra with the following thermal profile: 1 cycle of 95 ºC for 5 min (initial denaturation), followed by 45 cycles of amplification with denaturation at 95 ºC for 1 min, annealing at 36 ºC for 1 min and extension at 72 ºC for 2 min. The final extension was carried out at 72 ºC for 5 min. The amplified DNA were separated using electrophoresis unit (WIDE mini-sub-cell GT Bio-RAD) on 1% agarose containing ethidium bromide (0.5 µg/ml) at constant volt and determined with UV transilluminator.

Statistical analysis

The data obtained were analyzed with (SPSS) one-way ANOVA.

Table 1. Sequence of random primers used in RAPD-PCR

Primer Cod	Sequence (5'-3')
Primer 1	AGTCAGCCAC
Primer 2	TGCCGAGCTG
Primer 3	TCGGCGATAG
Primer 4	TCCGCTCTGG
Primer 5	TGTCATCCCC
Primer 6	AATGGCGCAG
Primer 7	CTCTCCGCCA
Primer 8	CTGAGACGGA
Primer 9	CAGCACCGCA
Primer 10	GTCCACTGTG

Results

Effect of plant extracts on germination and seedling growth

The effects of *Rhazya* extract concentrations on the germination of *R. sativus*, calculated as a percentage of their controls, are shown in (Table2). Generally no significant differences in the germination percentages occurred at low concentrations of *Rhazya* extract, but at 5% concentration a significant reduction was observed. Growth of *R. sativus* seedling treated with *Rhazya* aqueous extract, during the germination period, are shown in Table (2). The treatments at low concentrations of *Rhazya* extract increased the length of *R. sativus* radicle and plumule over the control, while the high concentrations produced significant growth reduction. The highest radicle and plumule lengths inhibition reached 0.21cm and 3.36 cm at concentrations 5% and 4%, respectively. Alternatively, the dry weight of radicle and plumule did not show significant difference except at 4% and 5% extract concentration which showed significant decrease in dry weight of radicle of treated plants.

Concerning root/shoot length and weight ratios, the results suggest that stimulatory and inhibitory effects of the plant extract of concentration (Figures 1&2). The low concentrations of *Rhazya* extract increased root/shoot length ratio at concentrations up to

0.1% then decreased at higher concentrations (Figure 1). Reduced root/shoot weight ratio of *R. sativus* was significant above concentration 0.5% of *Rhazya* extract (Figure2).

Table 2. Effect of different concentrations of *Rhazya stricta* plant aqueous extract on germination percentage and growth criteria of *Raphanus sativus*.

Extract Concentration (%)	Growth Criteria				
	% of Germination	Length of Radicle (cm)	Length of Plumule (cm)	Dry Weight of Radicle (gm)	Dry Weight of Plumule (gm)
0	100.00±0.00	12.21±3.07^{c}	6.46±0.90^{cd}	0.002±0.00^{ef}	0.006±0.00^{ab}
0.05	100.00±0.00	12.48±1.25^{c}	8.39±0.43^{ef}	0.001±0.00^{e}	0.006±0.00^{abc}
0.1	100.00±0.00	17.79±1.03^{d}	7.32±1.33^{de}	0.002±0.00^{f}	0.006±0.00^{ab}
0.5	100.00±0.00	11.87±4.46^{c}	10.55±0.08^{g}	0.001±0.00^{d}	0.008±0.00^{de}
1	95.00±0.00	4.38±1.77^{b}	8.77±0.86^{f}	0.001±0.00^{cd}	0.009±0.00^{e}
2	100.00±0.00	0.30±0.10^{a}	5.37±0.24^{bc}	0.001±0.00^{bc}	0.008±0.00^{cd}
3	100.00±0.00	0.22±0.09^{a}	4.28±0.38^{ab}	0.001±0.00^{b}	0.007±0.00^{bcd}
4	100.00±0.00	0.23±0.05^{a}	3.36±0.91^{a}	0.000±0.00^{a}	0.007±0.00^{abcd}
5	80.00±0.00	0.21±0.04^{a}	3.97±0.10^{a}	0.000±0.00^{a}	0.005±0.00^{a}

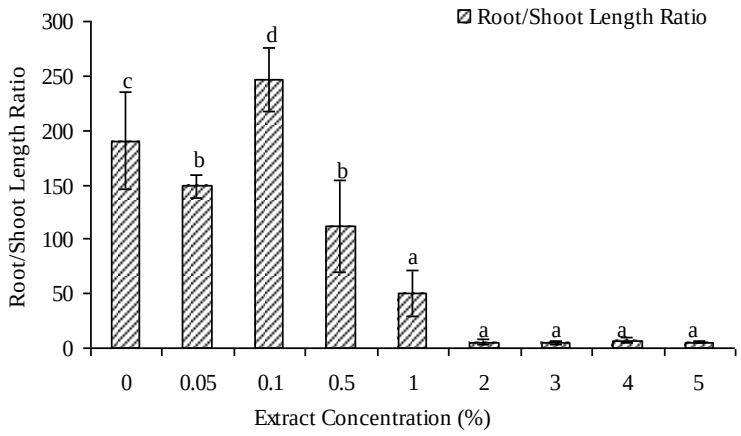

Fig.1. Effect of different concentrations of *Rhazya stricta* plant aqueous extract on root/shoot length ratio of *Raphanus sativus*.

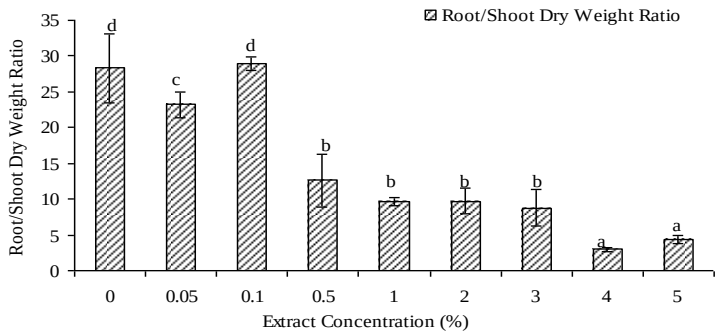

Fig.2. Effect of different concentrations of *Rhazya stricta* plant aqueous extract on root/shoot weight ratio of *Raphanus sativus*.

Effects on plant growth

In the pot experiment, the growth response of *R. sativus* at different concentrations as affected by *Rhazya* residue is shown in Tables (3-1&2). At 30 days old *R. sativus* shoot length decreased with the decrease of *Rhazya* residue compared to control, whereas at 60 days old, shoot length generally showed no constant trend with the increase of *Rhazya* residue concentration. At age of 30-days, the increase of *Rhazya* residue caused a significant decline in *R. sativus* root length at high concentration, while at 60 days old a significant decline was observed in all *Rhazya* residue concentrations. The 4 grams concentration of the residue caused the highest root length. Reduced root/shoot length ratio of *R. sativus* at ages of 30 and 60 days was significant in all *Rhazya* residue concentrations. The highest root/shoot length ratio inhibition at age of 30 days reached 51.40% at residue concentration of 16 grams, while at age of 60 days, the root/shoot length ratio reached 71.7% at residue concentration of 10 grams. The dry weight of *R. sativus* at age of 30 days did not show any positive or negative variation from the control treatment except at 10-12 and 16 grams residue concentration which showed a significant decrease in dry weight of treated plant. At age of 60-days, the increased *Rhazya* residue caused a significant decline in *R. sativus* dry weight particularly at 12 and 16 grams residue concentrations.

Table 3-1. Effect of different concentrations of *Rhazya stricta* plant residues on seedling growth of 30 days-old *Raphanus sativus* plant

Residue Concentration (g)	Age: 30 days			
	Shoot Length (cm)	Root Length (cm)	R/S Length Ratio (%)	Dry Weight (g)
0	6.50 ± 0.72^{cd}	4.16 ± 0.85^{bc}	64 ± 13.72^{ab}	0.052 ± 0.002^{d}
2	5.32 ± 1.51^{abc}	5.20 ± 1.25^{c}	110.8 ± 62.34^{c}	0.056 ± 0.002^{e}
4	6.04 ± 0.71^{bcd}	3.86 ± 1.17^{b}	64.6 ± 22.09^{ab}	0.064 ± 0.002^{f}
6	6.66 ± 0.83^{d}	4.04 ± 0.79^{bc}	60.8 ± 12.02^{ab}	0.054 ± 0.002^{de}
8	6.14 ± 0.63^{bcd}	4.14 ± 0.51^{bc}	67.40 ± 10.31^{ab}	0.053 ± 0.001^{de}
10	4.40 ± 0.22^{cd}	3.48 ± 0.60^{ab}	53.6 ± 8.84^{a}	0.042 ± 0.002^{c}
12	4.36 ± 0.32^{a}	4.06 ± 0.83^{bc}	93.4 ± 22.81^{bc}	0.033 ± 0.002^{b}
16	5.18 ± 1.08^{ab}	2.58 ± 0.69^{a}	51.40 ± 20.11^{a}	0.025 ± 0.003^{a}

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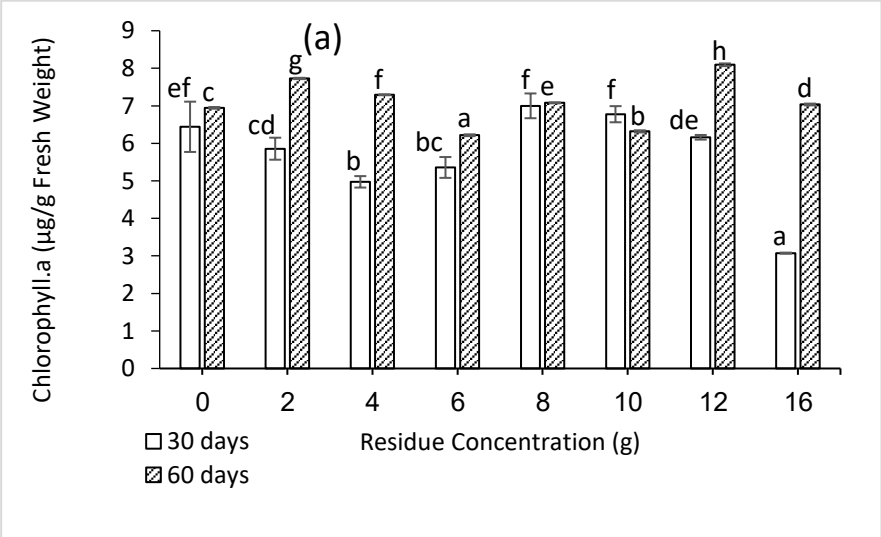
Table 3-2. Effect of different concentrations of *Rhazya stricta* plant residues on seedling growth of 60-days-old *Raphanus sativus* plant.

Residue Concentration (g)	Age: 60 days			
	Shoot Length (cm)	Root Length (cm)	R/S Length Ratio (%)	Dry Weight (g)
0	5.72 ± 0.4^{bc}	9.7 ± 1.51^{c}	170.33 ± 29.14^{d}	0.22 ± 0.002^{f}
2	5.7 ± 0.83^{bc}	6.82 ± 1.19^{b}	122.20 ± 30.22^{bc}	0.18 ± 0.002^{d}
4	6.66 ± 0.99^{c}	9.16 ± 0.74^{c}	140.87 ± 28.36^{cd}	0.21 ± 0.002^{e}
6	5.66 ± 0.82^{bc}	5.78 ± 0.63^{ab}	102.82 ± 8.78^{b}	0.18 ± 0.002^{d}
8	4.1 ± 0.44^{a}	5.7 ± 0.84^{ab}	141.04 ± 29.10^{cd}	0.14 ± 0.002^{c}
10	8.26 ± 0.87^{d}	5.86 ± 0.48^{ab}	71.7 ± 10.75^{a}	0.34 ± 0.002^{g}
12	5.1 ± 0.59^{b}	5.76 ± 0.69^{ab}	113.78 ± 14.90^{bc}	0.1 ± 0.002^{a}
16	5.7 ± 0.57^{bc}	5.42 ± 0.36^{a}	95.60 ± 8.68^{ab}	0.11 ± 0.003^{b}

Effects on photosynthetic pigments content

Changes in the various photosynthetic pigments in the shoot system of *R. sativus* for 30 and 60 days with different rates of *Rhazya* plant residue are shown in Figure (3). At age 30 and 60 days, Chlorophyll a doesn't show any significant difference except at 16 grams residue concentrations which shows significant decrease in chlorophyll a content after 30 days, while at age 60 days, chlorophyll a content increased by 12 grams residue concentration(Figure 3-a). Significant reduction in chlorophyll b content of *R. sativus* was detected by the effect of 10 - 12 and 16 grams residue after 30 day and at 60 days age, the chlorophyll b content increased by 6 grams residue concentration, while at 4 and 8 grams residue concentration, the chlorophyll b content of *R. sativus* significantly decreased compared with the control (Figure 3-b).

Concerning chlorophyll a/b ratio of *R. sativus* at 30 and 60-days age generally increased with increasing the concentrations of *Rhazya* residue (Figure 3-c). At 30 days age carotenoid of *R. sativus* showed a significant increase with increasing residue concentration, while at 60 days age an increase in carotenoid content was is highly marked at both 4 and 10 grams residue concentration (Figure 3-d).



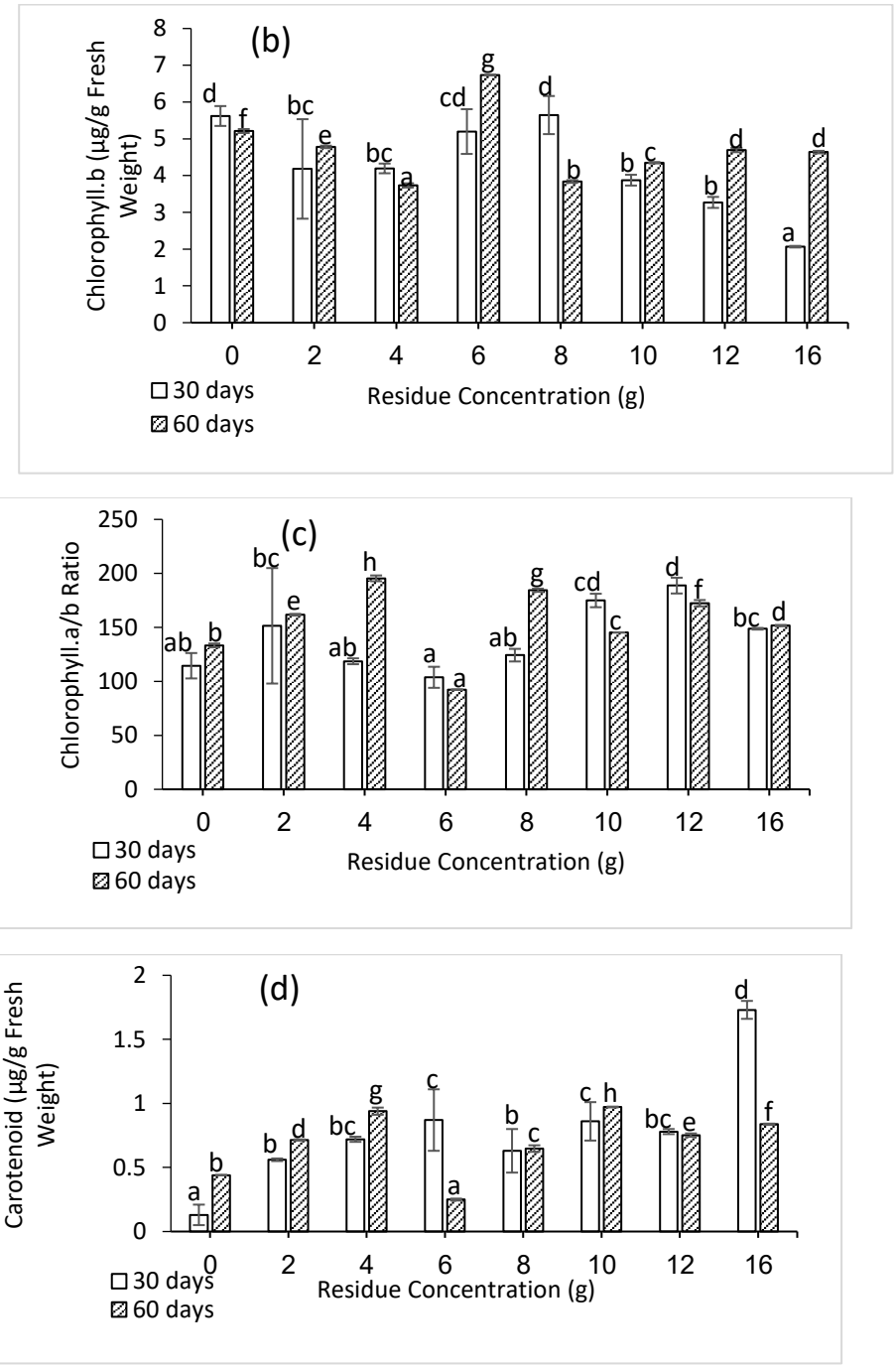


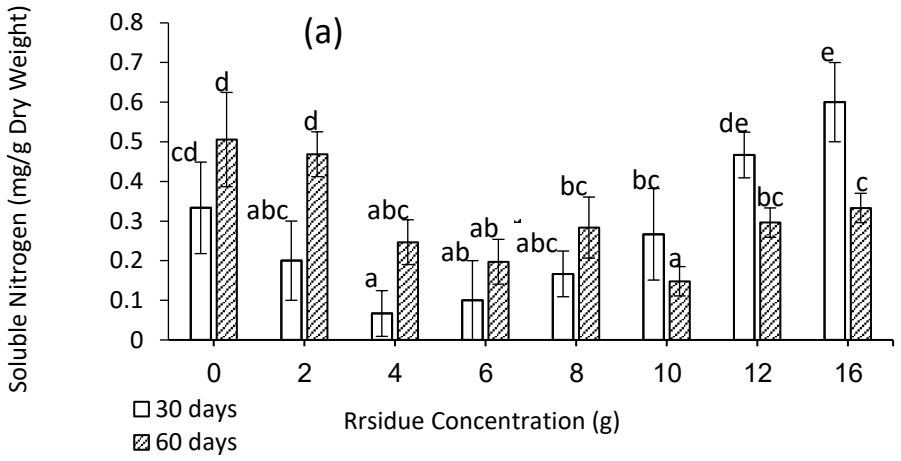
Fig.3. Effect of different concentrations of *Rhazya stricta* plant residues on the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid) of 30 and 60-days-old *Raphanus sativus*. Vertical bars are standard deviation of the mean.

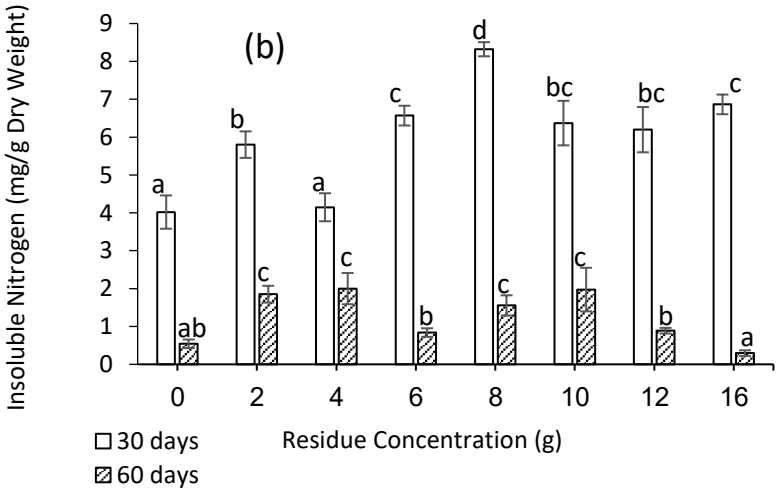
Effects on nitrogen content

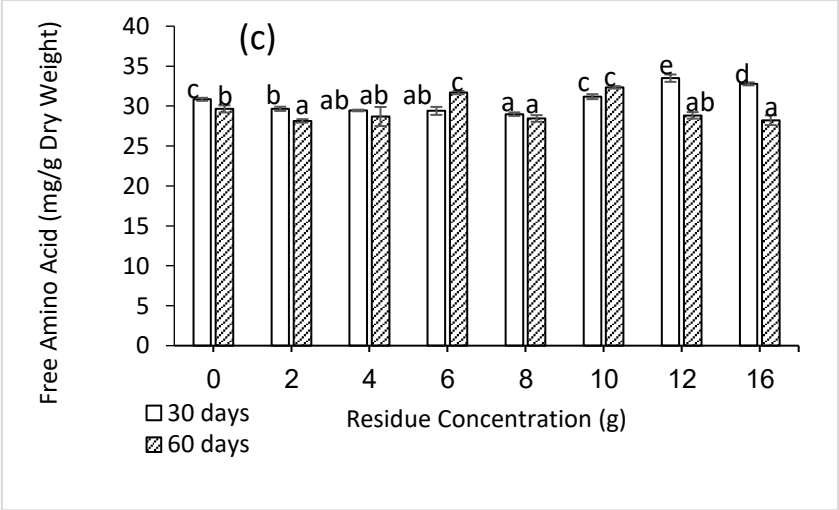
The change in the nitrogenous components of 30 and 60-days-old *R. sativus* in response to different *Rhazya* treatments are shown in Figure (4). At 30-days old, *R. sativus* soluble nitrogen decreased by 4 grams, while at higher *Rhazya* residue, the soluble nitrogen of *R. sativus* significantly increased to (o.60mg/g dry weight) compared with control treatment. In addition after 60 days the plant showed significant reduction in soluble nitrogen which is highly marked at 10 grams *Rhazya* residue concentration (Figure 4-a).

With respect to insoluble nitrogen 30 days age *R. sativus* plant generally increased with increasing the concentrations of *Rhazya* residue. After 60 days age, plants showed significant increase in insoluble nitrogen except at 16 grams residue concentration which showed a marked decrease in insoluble nitrogen of the treated plant as shown in (Figure 4-b). In contrast, *R. sativus* at age of 30 days showed a decrease in free amino acid at lower residue concentration while a significant increase was remarked with increasing residue concentration. After 60 days, plants did not show any significant difference except at 6 and 10 grams residue concentration which attained significant increase in free amino acid (Figure 4-c).

Crude protein of *R. sativus* at 30 days age increased with the increase of *Rhazya* residue. In addition at 60 days aged plant, crude protein showed significant increase that is highly marked at both 2 and 4 grams residue concentration, while 16 grams residue concentration showed significant decrease in crude protein (Figure 4-d).


(a)
Soluble Nitrogen (mg/g Dry Weight)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
cd
d
abc
d
a
abc
ab
ab
abc
bc
bc
a
de
bc
e
c
0
2
4
6
8
10
12
16
30 days
60 days
Rrsidue Concentration (g)


(b)
Insoluble Nitrogen (mg/g Dry Weight)
9
8
7
6
5
4
3
2
1
0
a
ab
b
c
a
c
c
b
d
c
bc
c
bc
b
c
a
0
2
4
6
8
10
12
16
30 days
60 days
Residue Concentration (g)


(c)
Free Amino Acid (mg/g Dry Weight)
40
35
30
25
20
15
10
5
0
c
b
b
a
ab
ab
ab
c
a
a
c
c
e
ab
d
a
0
2
4
6
8
10
12
16
30 days
60 days
Residue Concentration (g)

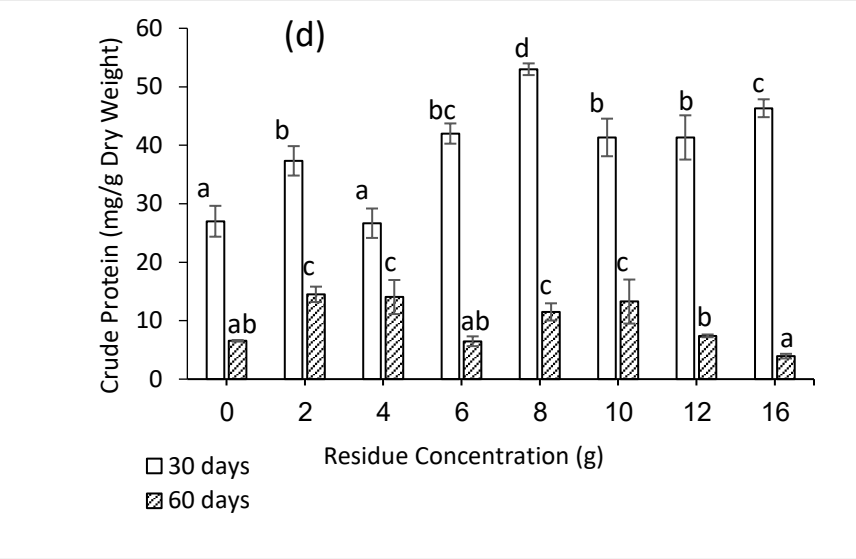


Fig. 4. Effect of different concentrations of *Rhazya stricta* plant residues on the nitrogen fractions (soluble and insoluble nitrogen, free amino acids and crude protein) of 30 and 60-days-old *Raphanus sativus*. Vertical bars are standard deviation of the mean.

Effects on RAPD profiles of DNA

The results of RAPD-DNA profiles of *R. sativus* plant are summarized in Tables (4-1&2). The changed bands observed in RAPD profiles (e.g. disappearance, appearance of bands, decrease and increase in band intensity in comparison to RAPD profiles of the control). Number of disappearing bands was observed with all concentrations by using primer 4. With primer 2, disappearance of bands was indicated at concentrations 4, 8, 12 and 16 grams *Rhazya* residue. In addition, primer 6 indicated disappearance of one band at concentration 8 grams *Rhazya* residue (Figure 5). Extra bands appeared with primer 1 one new PCR amplification products at concentration 10 grams *Rhazya* residue and one new band appeared with primer 2 at concentration 8 grams *Rhazya* residue, while with primer 3 two new bands appeared at concentration 6 grams *Rhazya* residue. One new band was observed at concentrations 4, 10 and 12 grams *Rhazya* residue with primer 6 and one new bands appeared by using primer 7 at concentrations 2, 4 and 6 *Rhazya* residue. Primer 8 indicted the appearance of one new band at concentrations 6, 10 and 16 grams

Rhazya residue and also by primer 9 one new bands was indicated at concentration 6 grams *Rhazya* residue (Figure 5). The increase in bands intensity were obvious at concentrations 6, 10 and 12 grams *Rhazya* residue for primer 1, 2, 6, 7, 9 and 10. In contrast, a decrease in bands intensity occurred in all concentrations but, were particularly obvious at concentration 8 grams *Rhazya* residue for primer 2, 3, 5 and 9 (Figure 5).

Table 4-1 Changes of bands in the shoot of *Raphanus sativus* treated with different concentrations of *Rhazya* residue.

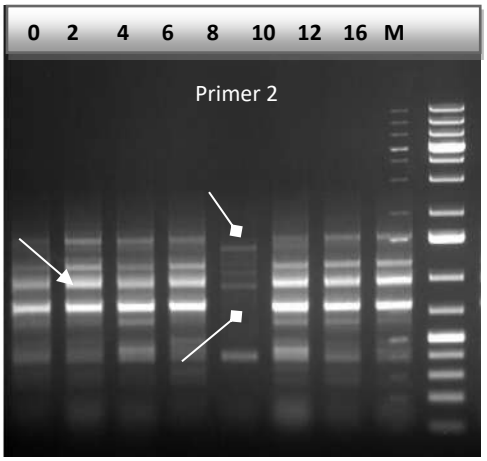
Primer	**Cont**	***Rhazya* residue (g)**																			
		2					**4**					**6**					**8**				
		a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	B	c	d	e
Primer 1	7	7	-	-	3	-	7	-	-	3	-	7	-	-	6	-	7	-	-	3	1
Primer 2	12	12	-	-	4	-	11	-	1	5	-	12	-	-	5	-	9	1	4	1	4
Primer 3	7	7	-	-	-	3	7	-	-	-	3	9	2	-	4	1	7	-	-	-	3
Primer 4	4	3	-	1	1	1	2	-	2	-	-	2	-	2	1	-	2	-	2	-	1
Primer 5	7	7	-	-	-	2	7	-	-	-	4	7	-	-	-	4	7	-	-	-	4
Primer 6	8	8	-	-	6	-	9	1	-	5	-	8	-	-	6	-	7	-	1	5	1
Primer 7	9	10	1	-	2	1	10	1	-	2	1	10	2	-	2	2	10	1	-	3	1
Primer 8	9	9	-	-	2	-	9	-	-	2	1	10	1	-	3	-	9	-	-	2	-
Primer 9	9	9	-	-	4	4	9	-	-	4	1	9	1	-	3	2	9	-	-	2	3
Primer 10	8	8	-	-	1	-	8	-	-	1	-	8	-	-	1	-	8	-	-	1	1
Total bands	80	80	1	1	23	11	79	2	3	22	10	82	6	2	31	9	75	2	7	17	19
b+ c		2					5					8					9				
b+ c+ d+ e		36					37					48					45				

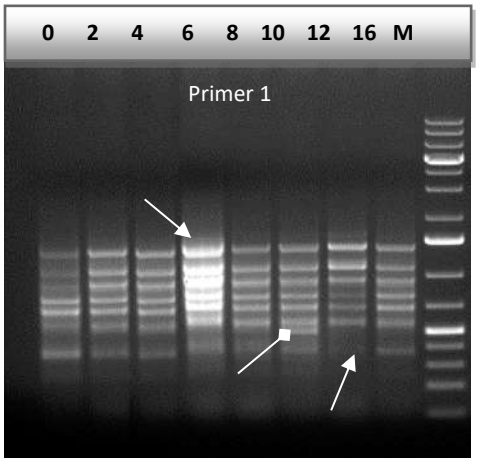
a:indicates number of band in treatment, b: appearance of new bands, c: disappearance of normal bands, d: increase in band intensities, e: decrease in band intensities. b+ c, denote polymorphic bands, and b +c + d+ e :varied band.

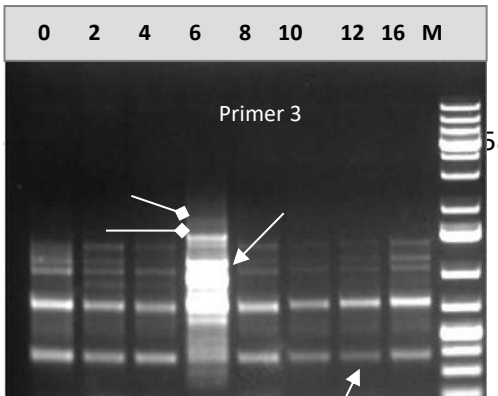
Table 4-2 Changes of bands in the shoot of *Raphanus sativus* treated with different concentrations of *Rhazya* residue.

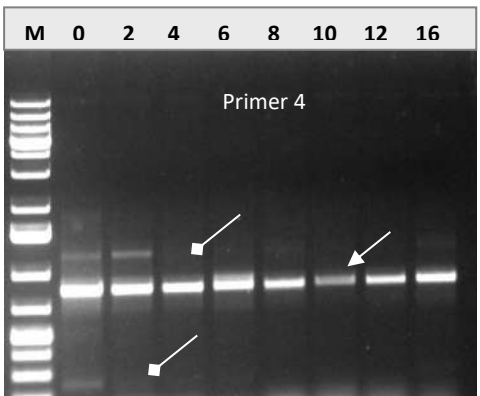
Primer	Control	*Rhazya* residue (g)														
		10					12					16				
		a	b	c	d	e	a	b	c	d	e	a	b	c	D	e
Primer 1	7	8	1	-	5	-	7	-	-	4	2	7	-	-	4	-
Primer 2	12	12	-	-	6	-	11	-	1	5	-	11	-	1	4	-
Primer 3	7	7	-	-	-	4	7	-	-	-	4	7	-	-	3	1
Primer 4	4	2	-	2	-	1	2	-	2	-	1	2	-	2	-	1
Primer 5	7	7	-	-	-	4	7	-	-	2	3	7	-	-	2	3
Primer 6	8	9	1	-	6	-	9	1	-	6	-	8	-	-	6	-
Primer 7	9	10	2	-	6	-	10	2	-	2	2	10	1	-	2	2
Primer 8	9	10	1	-	2	2	9	-	-	1	4	10	1	-	2	2
Primer 9	9	9	-	-	3	2	9	-	-	3	2	9	-	-	3	2
Primer 10	8	8	-	-	1	1	8	-	-	1	1	8	-	-	1	-
Total bands	80	82	5	2	29	14	79	3	3	24	19	79	2	3	27	11
b+ c		7					6					5				
b+ c+ d+ e		50					49					43				

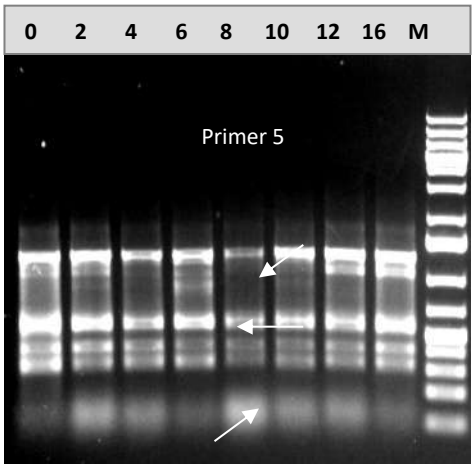
a:indicates number of band in treatment, b: appearance of new bands, c: disappearance of normal bands, d: increase in band intensities, e: decrease in band intensities. b+ c, denote polymorphic bands, and b +c + d+ e :varied band

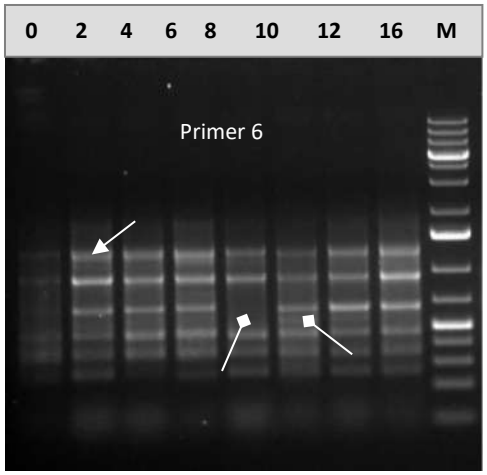


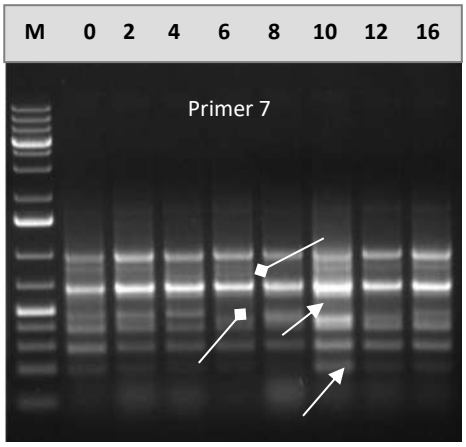


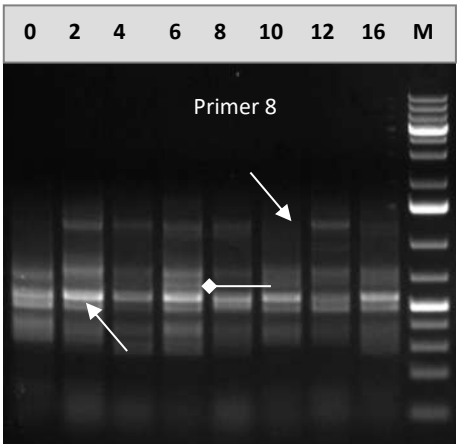


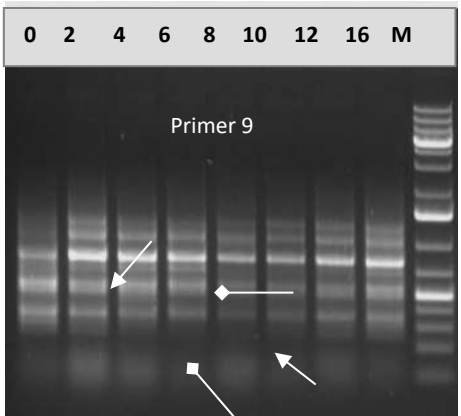



0 2 4 6 8 10 12 16 M
Primer 5


0 2 4 6 8 10 12 16 M
Primer 6


M 0 2 4 6 8 10 12 16
Primer 7


0 2 4 6 8 10 12 16 M
Primer 8


0 2 4 6 8 10 12 16 M
Primer 9

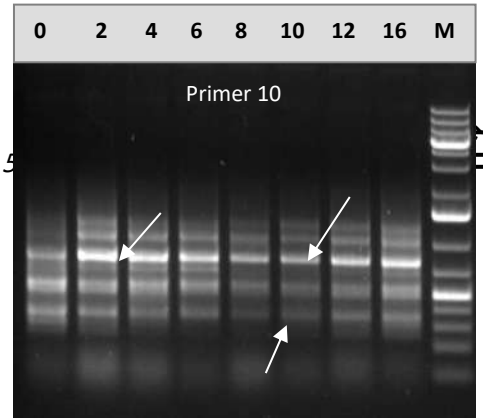

0 2 4 6 8 10 12 16 M
Primer 10

Fig.5. RAPD profiles of genomic DNA of *Raphanus sativus* treated by various concentrations *Rhazya* residue generated using different of RAPD primers as indicated on each gel. ⟶ appearance of new band band and disappearance of normal band ⟶ varied band intensities.

Discussion

The results of the present study showed that the aqueous extracts of *Rhazya stricta* differed in their effects on seedling and adult plant growth, photosynthetic pigments, nitrogen content and RAPD-DNA profiles of Radish (*Raphanus sativus*) plant. The *Rhazya* extract was not significantly affecting on germination percentage of *R. sativus*. The results showed that allelochemicals in the extract of *Rhazya* could have harmless effect on seed germination of *R. sativus*. This result agrees with the earlier study of (Moosavi *et al*,. 2011,p 115) who demonstrated that allelopathic effect of different concentrations of water extract of *sorghum* was not significant for germination percentage of *Vigna radiata L*.

The extracts of *Rhazya* stimulated significantly the lengths, weight and root/shoot length and weight ratios of *R. sativus* particularly at the low concentrations, whereas at high concentrations produced inhibitory effect. This indicated that allelochemicals in the extract of *Rhazya* may have stimulating effect on seedling growth of *R. sativus*. On the other hand, the inhibition was correlated to the concentration of the inhibitory

chemicals present in high concentrations for *Rhazya* extract. Similarly, (Mutlu & Atici,. 2009, p 90) demonstrated, both root and leaf extracts significantly increased the seedling growth of wheat, especially at the lower concentrations. The biological activity of the identified allelochemicals from *Vulpia myuros* toward test plant was stimulatory at low concentrations, and inhibitory at high concentrations (An *et al*,. 2001, p 383).

The effect on growth suggests that leaves and stem of *Rhazya* can act as a source of allelochemicals after decomposition that in-turn negatively affects the neighboring or successional plants. The observed phytotoxicity difference of *Rhazya* may be attributed to the presence of variable amount of phytotoxic substances in different parts that leach out under natural conditions. Some modern investigations indicating the allelopathic/ phytotoxic determine of aqueous extracts of weeds contain include *Raphanus raphanistrum* (Norsworthy,. 2003, p 307), *Andrographis paniculata* (Alagesaboopathi,. 2011, p 147). These studies strongly showed the release of phototoxic chemicals during the preparation of aqueous extracts.

In pot experiment, incorporation of *Rhazya* residue into the soil at the low concentrations, the dry weight of *R. sativus* was stimulated particularly at early stages of growth. However, the inhibition of root to shoot length ratio and dry weight was more pronounced at late age stages than at the early stage. Conversely, increasing the rate of *Rhazya* residue caused an inhibition in growth of radish at different ages. The inhibition of cell elongation may be related to the direct action of allelochemicals by interfering with cell division either directly or through interaction with hormones. These results are in accordance with (Al-Wakeel *et al*,. 2007, p 413) who demonstrated the stimulation in root and shoot lengths of 45-day-old pea irrigated with *Acacia nilotica* leaf extract, while the higher concentration were inhibitory. On the contrary with (Abu-Romman,. 2011, p 948) showed that the growth of pepper was significantly inhibited with increasing of *Achilliea* leachate concentration.

The residue of *Rhazya* showed both inhibitory and stimulatory effects on photosynthetic pigments of *R. sativus* at different ages.

Chlorophylls are the core component of pigment protein complexes embedded in the photosynthetic membranes and play a major role in the photosynthesis. Any changes in chlorophyll content are expected to bring about change in photosynthesis (Reigosa,. 2006, p 315). The inhibition in Chl a and Chl b were previously reported as a result of allelochemical stress (Singh *et al*,. 2009, p 163) or may be due to the inhibition of chlorophyll biosynthesis or stimulation of chlorophyll degradation or both processes (Yang *et al*,. 2002, p 303). Moreover, (Siddiqui,. 2007, p 306) reported a reduction in chlorophyll content of *Vigna mungo* due to the allelochemicals present in leachates of black pepper which possibly target enzymes responsible for the conversion of porphyrin precursors.

Based on the results, a significant decrease in nitrogen content of *R. sativus* including total amount of free amino acid, soluble and insoluble nitrogen and crude protein were related to the age. This could be due to the higher levels of *Rhazya* allelochemicals, which have harmful effect on nitrogen metabolism (Reigosa,. 2006, p 320). According to the allelopathy definition, it is so evident that allelochemicals could affect all phases of nitrogen cycle involved in plant or microorganisms. When plants take up nitrate, they must use energy to convert it to ammonium form before it can be used (Reigosa,. 2006, p 321). The growth reduction due to missing energy could be an argument for nitrogen reduction in seedlings which treated by allelochemicals, also loosing of nitrogen content in some seedling, may be occurred by limiting or reducing some key factors in nitrogen metabolism such as nitrate reductase and glutamine synthetase (Nie,. 2005). In contrast, the lower concentrations of *Rhazya* residue stimulated the contents of insoluble nitrogen and crude protein of *R. sativus* in the late stage. This effect could be related to the interaction of *Rhazya* allelochemicals with nitrogen uptake and metabolism. Similarly, (Al-Wakeel *et al*,. 2007, p 416) demonstrated that the content of total nitrogen (their insoluble form), increased with lower *Acacia* residues whereas all nitrogen fraction declined by increasing *Acacia* residues.

Understanding the mechanisms by which higher plants perceive environmental stimuli is of vital importance to modern molecular biology. In practice, the key point to agriculture is how to regulate the harmonious relationship between soil-environment and crops and make the best of physiological potential of crops (Gang *et al*,. 2007, p 117). To some extent, plants could overcome environmental stress by developing efficient and specific physio-biochemical mechanisms (Sandalio *et al*,. 2001, p 2122). In "genetic-ecotoxicology" or "eco-genotoxicology", the effective evaluation and proper environmental monitoring of potentially genotoxic pollutions will be improved with development of sensitive and selective methods to detect toxicant-induced alterations in the genomes of a wide range of biota (Liu *et al* ,. 2007, p 1160). As reported by many researchers (Atienzar and Jha,. 2006, p 98) the alteration in DNA fingerprinting is a useful biomarker in eco-genotoxicology.

In the present study, DNA damage induced by *Rhazya* residue treated *R. sativus* seedling was reflected by changes in RAPD profiles: variation in band intensity, disappearance of bands, and appearance of new PCR products occurred in the profiles. These results indicated that genomic stability in *R. sativus* seedling was significantly affected by *Rhazya* residue stress. Modifications of band intensity and lost bands are likely to be due to one or a combination of the events (changes in oligonucleotide priming sites due to genomic rearrangements and less likely to point mutation and DNA damage in the primer binding sites, interactions of DNA polymerase in *R. sativus* seedling with damaged DNA). The disappearance of normal band (band loss) may be related to the events such as DNA damage (e.g. single- and double-strand breaks, modified bases, abasic sites, oxidized bases, bulky adducts, DNA protein cross links), point mutation and/or complex chromosomal rearrangements induced by genotoxins (Atienzar *et al*,. 2002, p 160). The new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage (Atienzar & Jha,, 2006, p 99).

Conclusion

The allelopathic activity of *Rhazya stricta* is depending on the amount and type of allelochemicals released from the decomposed shoot, as well as the uptake of these compounds by plant roots of the target species

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الأثر الأليلوباثي لمسحوق نبات الحرمل (*Rhazya stricta*) علي نبات الفجل (*Raphanus sativus*)

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الملخلص

تمت دراسة الإمكانية الأليلوباثية لنبات الحرمل (*Rhazya stricta*) علي الفجل تحت الظروف المعملية والطبيعية. أوضح المستخلص المائي لنبات الحرمل تأثيرا مثبطا علي نمو بادرات نبات الفجل خاصة في التركيزات العالية بينما كان التأثير ضعيفا علي نسبة الإنبات. كما أوضحت معاملة التربة بمسحوق نبات الحرمل تحت الظروف الطبيعية تأثيرا مثبطا علي طول جذر النبات والوزن الجاف إضافة إلي نسبة طول الجذر/ الساق وفي مختلف الأعمار. كما أظهر مسحوق الحرمل آثار إيجابية علي أصباغ البناء الضوئي لنبات الفجل خاصة الكاروتينات ونسبة الكلوروفيل وقد ارتفع محتوي النبات من الأحماض الأمينية الحرة ومحتوي النيتروجين القابل وغير القابل للذوبان والبروتين بنسب واضحة في مراحل النمو المبكرة عمر(30 يوم)، بينما ازداد محتوي النبات من النيتروجين القابل للذوبان وكان ذلك في مراحل النمو المتأخرة (عمر 60 يوم).

أظهرت تحاليل RAPD-DNA التأثير الهام لمسحوق نبات الحرمل علي نبات الفجل من حيث الاختلاف في كثافة الأحزمة، اختفاء الأحزمة وظهور أحزمة جديدة.
الكلمات الدالة: المواد الأليلوكيميائية، المستخلص المائي ، أصباغ البناء الضوئي، تحاليل RAPD-DNA، مسحوق.